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THE ROLE OF ENDOGENIC PYROGEN IN IMMUNOGENESIS

Report I.

The Influence of Endogenic Pyrogen on Antibody Formation and the Intensity of Protein Synthesis in the Organism

[Following is the translation of an article by I. Y. Uchitel, E. L. Khasman and G. V. Karnoz, Institute of Epidemiology and Microbiology imeni Gamaleya, AMN, USSR, Moscow, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii, Vol 42, 1965, pages 3-7. It was submitted on 3 Sep 1964.]

In recent years a great role in the origin of fever has been attributed to endogenic pyrogens which develop in the organism itself under the influence of diverse stimuli.

After Beeson in 1948 isolated the thermogenic component from polymorphonuclear leukocytes numerous investigations were made. These testified that endogenic pyrogen plays a central role in the pathogenesis of fever (Benett and Beeson, 1953; Vud, 1958; Veselkin, 1963). However, the majority of the investigators studied the conditions for the development of endogenic pyrogen in the organism, its connection with leukocytes, focus of application, and its physical and chemical properties. This disregarded the question of its importance in the overall system of defense mechanisms in the organism, though it is known that fever is one of the most important companions of the infectious process and may exert a specific influence on immunogenesis.

Experiments conducted by us earlier (Uchitel and Khasman, 1964) showed that typhoid endotoxin sharply increases the intensity of protein synthesis in an organism, which apparently explains the stimulation of antibody formation by it.

Since at the present time the opinion prevails that the pyrogenic action of bacterial endotoxins is realized to a considerable degree through endogenic pyrogens, the assumption is emerging that their stimulation of protein synthesis and antibody formation also depend on the intensified production of endogenic pyrogens caused by them.

At the present time we have undertaken the task to study the influence of endogenic pyrogen on the production of antibodies and

protein synthesis in a number of organs and tissues in the body. We proposed that this would help in an understanding not only of the mechanism of stimulation of antibody production by endotoxins but also the role of febrile reactions in immunogenesis.

The experiments were carried out on chinchilla rabbits weighing 2-2.5 kg. Endogenic pyrogen was obtained by the method described by Bennett and Beeson (1953). Over a period of 3-3½ hours, using the drop method, we introduced into the abdominal cavity of the rabbits 350 ml each of physiological solution which contained from 50 up to 100 AU of penicillin. Then the animal was tied on the side for 3 more hours, after which the abdominal cavity was opened and the turbid fluid which had accumulated in it was drawn off. From a rabbit we obtained on the average of about 100 ml of exudate. The number of leukocytes was determined immediately. Usually it fluctuated from 15,000 to 20,000 cells in 1 ml. The leukocytes were extracted from the exudate by centrifuging. The cell-less exudate in which the endogenic pyrogen is contained was stored in a refrigerator for no more than 48 hours. The leukocytes were washed of fragments of exudate by repeated centrifuging with physiological solution. Then they were ground in a mortar with physiological solution, added on the basis of 1 ml per 200,000 cells. They were centrifuged for 20 minutes at 2,000 rpm. The cell fragments were discarded and the clear extract of leukocytes was used in further tests, since it showed a clearly expressed pyrogenic action.

Thus all the subsequent investigations were made with two preparations - cell-less exudate and an extract of leukocytes.

For determining the pyrogenic effect each series of preparation was administered intravenously to the rabbits, exudate on a basis of 10 ml and extract on the basis of 2 ml. The body temperature of the animals was measured every hour for 4 hours.

For studying the influence of endogenic pyrogens on antibody formation we used the typhoid vaccine as the antigen. It was administered intraperitoneally in a single dose of 500 million microbial cells simultaneously with the intravenous administration of the preparations of endogenic pyrogens being investigated. The blood for determination of titers was taken on the 3rd, 5th, 7th, 10th, and 15th days.

The intensity of protein synthesis in the various organs and tissues of the organism was judged by the extent of incorporation of labelled amino acid - glycine- C^{14} - in the protein. This was administered to the animals 4 hours after the intraperitoneal administration of the cell-free exudate or the extract of leukocytes, that is, at the moment of an increase of body temperature. The glycine- C^{14} was administered on the basis of 20,000 pulses per one gram of animal weight. The animals in a control group received only glycine- C^{14} . The animals were sacrificed in 18-24 hours.

The total proteins of the blood serum, its albumen and globulin fractions, and also proteins in the organs (liver, spleen, suprarenal glands and lymph glands) after their homogenization were precipitated with trichloroacetic acid to end concentration equal to 5%, washed of free radioactivity, and dried according to a previously described method (Uchitel and Konikova, 1955). For the purpose of removing the serum albumens and globulins we used a modified method of Korner and Debro (1958). Calculation of radioactivity was done in 2 mg of dry protein in an end-window counter. For judging the reliability of the investigation results we determined the probability of possible error in the evaluation of the results (P) according to the Student table.

It turned out that both the cell-less exudate and the extract of leukocytes show an expressed pyrogenic action. The body temperature of animals begins to rise already in an hour after their introduction. After 2 hours the temperature reaches a maximum and remains at the level attained for 4 hours, after which a lowering begins. After 24 hours the temperature is normal. However, in spite of the same type pyrogenic effect in subsequent experiments the cell-less exudate turned out to be somewhat more active than the extract of leukocytes. Thus, following the simultaneous administration of a small dose of vaccine both preparations stimulated the formation of antibodies, but in the animals which had received the extract of leukocytes the stimulation was short-lived and already on the 7th day the titers of antibodies in them were no different than the antibody titers in the control group, while in the group of animals which received cell-less exudate the antibody titers were higher than in the control group in all the periods of investigation.

During a study of the intensity of the synthesis of nonspecific proteins from an organism after the administration of endogenic pyrogens it was revealed that at the moment of increased temperature (after 4 hours) there was a considerable increase in the intensity of labelled amino acid incorporated in the protein from the lymph glands and the suprarenal gland (see table).

The results testify that endogenic pyrogens found in the leukocytes, together with an increase of body temperature, stimulate the synthesis of protein in the lymphatic apparatus and the suprarenal glands. Here the most expressed increase in the incorporation of labelled amino acid in protein is caused by the exudate which has been freed of leukocytes.

On the drawing, which presents data on the influence exerted by the cell-less exudate on the body temperature, intensity of protein synthesis in the lymphatic apparatus and the suprarenal glands, and on the production of antibodies it is clearly evident that already in the first hours after introduction of endogenic pyrogen not only is there an increase of body temperature but there is an intensification of protein synthesis. The administration of vaccine at this time is accompanied by an increase of antibody production.

Intensity of incorporation of glycine- C^{14} in the protein of an organism after introduction of preparations containing endogenic pyrogen (in pulses/min per 2 mg of protein)

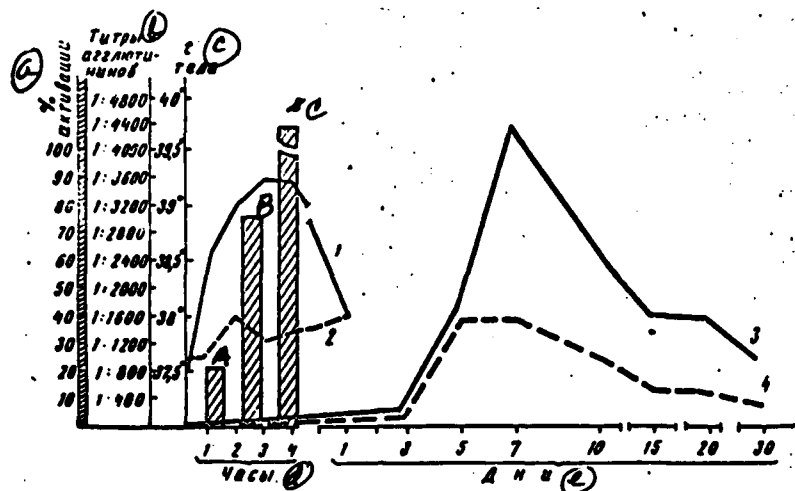
Препарат	Группы животных	Число животных	Температура		Радиоактивность белков сыворотки				Радиоактивность белков органов							
			до опыта	через 4 часа после опыта	сыворотка	альбумин	глобулин	сыворотка	сыворотка	надпочечники	селезенка	печень	селезенка	надпочечники	печень	селезенка
Бесклеточный эксудат	Подопытные	4	38,3°	39,1°	80	30	91	45	67	86	57	44	56			
	Контрольные	4	—	—	74	26	97	45	56	49	45	21	35			
	% активации				0	0	0	0	20	75	27	10	60			
Экстракт лейкоцитов	Подопытные	4	38°	39,2°	78	26	93	49	76	74	55	33	41			
	Контрольные	4			74	26	97	45	56	49	49	21	35			
	% активации				5	0	0	0	35	51	12	57	16			
	Величина P								0,1	0,01	<0,01	<0,02	0,01			
									>0,001	>0,001	>0,05	>0,05				

Key: (a) Preparation; (b) Group of animals; (c) Number of animals; (d) Temperature; (e) prior to test; (f) 4 hours after test; (g) Radioactivity of serum proteins; (h) total protein; (i) albumen; (j) globulin; (k) Radioactivity of proteins from organs; (l) liver; (m) spleen; (n) suprarenal glands; (o) retroperitoneal lymph node; (p) mediastinal lymph node; (q) popliteal lymph node; (r) Cell-less exudate; (s) Extract of leukocytes; (t) (from top to bottom): test; control; % activation; value of P.

The data obtained testifies that the action of endogenic pyrogens which are liberated by polymorphonuclear leukocytes is not limited to an increase of body temperature but is extended also to a number of processes taking place in the body. The increase of body temperature is only one of the links of the complex chain of interdependent reactions in the body.

In our experiments the exudate taken from the abdominal cavity turned out to be more active after the leukocytes were extracted from it than the extract which was obtained from these same leukocytes. This may be connected with the fact that in exudate which has not been subjected to any additional treatment the endogenic pyrogen is preserved in the least changed form.

Our experiments showed that endogenic pyrogens of leukocyte origin possess the capacity for nonspecific stimulation of antibody formation.



Change in the intensity of nonspecific and specific proteosynthesis under the influence of endogenic pyrogen.

A - spleen; B - suprarenal gland; C - regional lymph node. Body temperature of rabbits which had received endogenic pyrogen (1), control rabbits (2), titer of agglutinins in rabbits which had received vaccine and endogenic pyrogen (3), in control rabbits which had received only vaccine (4).

Key: (a) % activation; (b) Titers of agglutinins; (c) t, body; (d) Hours; (e) Days.

An analogous property, as this was established long ago, is possessed by various exogenic pyrogens - endotoxins of Gram-negative bacteria (Johnson et al., 1956; Kind and Johnson, 1959; Farthing, 1961; Farthing and Holt, 1962).

At the present time a number of factors are known which testify that the fever caused by endotoxins is caused by the interaction of endotoxin with leukocytes, as a result of which endogenic pyrogen is liberated and this causes the appearance of fever. And this makes it possible to assume that the nonspecific stimulation of antibody formation which is caused by endotoxins is connected, at least partially, with the appearance of endogenic pyrogens in the body, especially as the changes in the intensity of protein synthesis taking place under the influence of endogenic pyrogens are analogous to the changes caused by endotoxin from typhoid bacteria. Thus in the body of an animal the introduction of endogenic pyrogen stimulates the intensity of incorporation of labelled amino acid in the proteins of the lymphatic system and the suprarenal glands - organs which take part in antibody formation or its regulation. Such a stimulation of protein synthesis was observed by us earlier following administration in an organism of various non-specific stimulators of antibody formation (Uchitel and Khasman, 1964).

In the end result our data show that the fever which accompanies infectious diseases promotes favorable conditions for the carrying out of self-defense by an organism thanks to the stimulation of antibody formation which in turn is due to the endogenic pyrogens of cellular origin which are formed during infection.

Conclusion

When administered with vaccine the endogenic pyrogens which are liberated by polymorphonuclear leukocytes contribute to the capacity for nonspecific stimulation of antibody formation with a simultaneous increase in the intensity of protein synthesis in the lymphatic system and suprarenal glands.

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